INFLUENCE OF THIRTEEN DIFFERENT STRAINS OF ERICOID ENDOMYCORRHIZAE ON ROOTING AND GROWTH OF MICROPROPAGATED AZALEA MOLLIS

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Abstract

In the wild, most of the *Ericaceae* are mycorrhized by typical ericoid endomycorrhizae thus overcoming the absence of root hairs and the difficulty of absorption of some nutrients in low pH soils. Many authors studied the relationship between root colonization and soil composition but only a few of them took in consideration the species of the fungi mycorrhizing the roots. Our previous works on *Vaccinium* pointed out the effects of different fungal strains on rooting and growth of in vitro produced plants. The aim of this research was to study the effect of the inoculum with different fungal strains on rooting, acclimatization and growth of the deciduous Azalea mollis, cv Glowing Embers (*Rhododendron japonicum*), micropropagated in vitro. Sterile microcuttings, planted on a sterilized turf substrate, were inoculated with *Hymenoschiphus ericae,* seven different strains of *Oidiodendron maius*, and some unidentified sterile mycelia, by superimposing the rooting medium on a pure fungal culture in agar. Rooting time and percentage was checked. Plant height and rosette diameter, leaf and shoot number of each plant were measured one year after transferring to ex vitro conditions. All tested strains were able to infect the roots of the azalea, but different effects were shown on microcutting rooting time and percentage. *Oidiodendron* strains were generally more effective than *Hymenoschiphus* on growth of established plantlets.

INTRODUCTION

Acclimatization may be a bottleneck in the commercial micropropagation of several woody species. This is specially due to plantlets showing altered morphology and physiology as cause of the peculiar in vitro environmental conditions. In vitro grown plantlets anomalies and their occurrence has been widely reviewed (Hazarika, 2006). In the case of *Rhododendron* losses of 20% to 40% has been addressed to plantlet acclimatization stress (Anderson, 1978). In the wild, most of the *Ericaceae* are mycorrhized by typical ericoid endomycorrhizae thus overcoming the absence of root hairs and the difficulty of absorption of some nutrients in low pH soils usually not suitable for most of the plant species. Many authors studied the relationship between root colonization and soil composition but only a few of them took in consideration the species of the fungi mycorrhizing the roots. Some attempts to improve acclimatization by inoculation with ericoid mycorrhizal fungi have been reported but the results are still controversial as not always those plants artificially inoculated with endophytic symbiont strains show better performances in term of acclimatization and subsequent growth. This indicates the need to select within the ericoid endomycorrhizae only those strains stimulating significant plant growth both during acclimatization, and possibly also latter on in the field, to be used in artificial inoculations (Vosatka et al, 2000). Our previous works on *Vaccinium* pointed out the different effects of different fungal strains on rooting and growth of in vitro produced plants by using the 'two-step indirect method' (Eccher and Noè, 2002).This method represents the best procedure to inoculate *Vaccinium* micropropagated plantlets to get high efficiency of root cell infection; in addition infected roots are allowed to develop in a more suitable substrate as compared with agar that is

considered responsible for several anomalies and impairment of in vitro formed root system (Zimmerman, 1988).
The aim of this research was to study the effect of the inoculum with different fungal strains on rooting, acclimatization and growth of the deciduous Azalea mollis, cv Glowing Embers (*Rhododendron japonicum*), micropropagated in vitro by using the 'two-step indirect method'.

MATERIALS AND METHODS

Sterile microcuttings of the deciduous Azalea mollis, cv Glowing Embers, routinely maintained on proliferation medium Economu & Read (1984) added with 18,7 μM 2 iP,were planted on a sterilized turf substrate (two-step indirect method) as described below. Fungus was inoculated in agar medium composed of malt extract (2% w/v), pH 4,5, and shoots were put for rooting on a layer of sterilized peat superimposed after 20 days, when the mycelium was developed. The following mycorrhizal strains were used: *Hymenoscyphus ericae* (HE) isolated on *Calluna spp*. in England; three sterile non-classified mycelia isolated on *Calluna spp*. (G1, G2 and PS4) in Italy; two selection of sporing fungi *Oidiodendron maius*, one isolated on *Calluna spp*. in Italy (OME) and one isolated on *Vaccinium spp*. in Poland on either Zn-contaminated soil (OMZ) and Cd-contaminated soil (OMC) or not contaminated soil (OMA); three more selections of *Oidiodendron maius* (OM 89-91) were isolated in Canada; two sterile mycelia (D8 and D9) isolated on *Vaccinium spp*. in France. Interaction between cultivar and fungal strain was ascertained by preliminary studies. All the fungi were supplied by the Department of Biology of the University of Turin.
Axenic shoots of 1.0-1.5 cm length were transferred to 500 cm^3 transparent boxes (Green-boxes, Duchefa) containing the fungus and the layer of superimposed sterilized peat. In each box 13 shoots were placed and four boxes for each fungal strain and the control were considered. Two different control plants were used: in one case shoot base was dipped for 5 seconds in a 50:50 (v/v) hydroalcoholic solution of 1000 mg l^{-1} IBA(control + IBA). In the other case shoots were dipped in a 50:50 (v/v) hydroalcoholic solution without IBA addition (control). In both controls the culture medium was the same as above reported for the inoculation trials. All cultures were routinely maintained at 23°C ± 1°C, under *Philips TDL 33* cool white fluorescent tubes, under irradiance of about 31 μmol s^{-1} m^{-2} and 16 h photoperiod. Rooting was checked after 90 and 120 days respectively. Plantlets obtained were transplanted in pot, 5,0 cm Ø, in sterilised peat-perlite substratum and acclimated in greenhouse. As plants were grown they were transplanted in larger pot (7x 7 x 7 cm) in peat-perlite substratum. Plant survival was scored after two months from transferring to ex vitro conditions and at the same time and ten months later plants were also assessed for: height, rosette diameter, leaf and shoot number. Plants were allowed to grow during winter by keeping them in glasshouse. Data were analysed by one-way Anova and the differences contrasted using the Tukey's test. Statistical analysis was performed at 5 % level using using SPSS 11.0 per Windows package by SPSS inc. Plant survival data were analyzed according to the $\chi 2$ test at **0.01 level

RESULTS

Shoots were able to root at a different extent depending on the treatment (tab1). Ninety days after the inoculation OMZ induced rooting in the 88.5% of the infected shoots this being the highest percentage within all the tested strains, significantly higher than that obtained with the control, 70.0%. On the other hand G2 scored the lowest percentage, 23.1 %, significantly lower in respect to the control plus IBA. After 120 days, when rooting final data record was done, shoots inoculated with PS4 and D9 scored the lowest rooting percentage, 44.2 %, while the results obtained with the other treatments did not significantly differ in comparison with the controls, a part for G1 that was significantly lower, 62.9%, than control plus IBA, 96.1 %. Referring to ex vitro plant survival an increase in survival percentage was recorded for OMC, 97.4 %, and OMA, 94%, as compared to the control, 70%.

Among the fungal strains which in the nursery stimulated plant initial growth in terms of height such as OMC,OM 89, OM 90 and G2 (tab.2), only G2 kept up this role after one year while in the case of OM 90 a significant decrease in plant height was recorded as compared to both the controls (tab.2). Four fungal strains OM 90, HE, D8 and D9 showed an inhibiting action on plant lengthening after one year whereas only one strain, OMA, enhanced this parameter, 16.8 cm, in comparison to the control, 14.3 cm. To get more information on fungal effect on plant vegetative vigour we also assessed canopy (rosette) diameter in horizontal projection. After two months eight fungal strains out of thirteen induced a reduction of rosette diameter and the remaining five did not cause any significant difference as compared with the two controls (tab.3); once again this behaviour was not predictive of what would have happened ten months later in fact, after one year no differences could be seen between treatments and controls a part for D9 which enhanced rosette growth scoring the significantly highest diameter (15.5 cm). Concerning shoot number (tab.4), at the beginning OME showed the highest mean number, 1.7, but later on, after one year, a general recover in terms of new shoots production flattened any statistical difference a part for D8 which gave the lowest shoot mean number, 2.0.

CONCLUSIONS

In this work the effect of mycorrhizal fungi on rooting, ex vitro survival and subsequent growth of Azalea mollis, cv Glowing Embers was shown. As regarding rooting the most relevant role exerted by the tested fungi seemed to be a detrimental effect on rooting efficiency as demonstrated by the infection with fungal strains PS4 and D9. *Oidiodendron maius* among the tested strains had some positive effects on survival and subsequent plant growth but, in disagreement with Jansa, J. and Vosátka M. (2000) did not stimulate rooting. The same Authors reported that a large amount of fungal strains has to be checked prior finding those which exert a beneficial role on plant growth; this low efficiency is also confirmed by the results of our work. Moreover our finding that some strains have even an inhibiting effect on post vitro growth makes the identification of beneficial strains an aspect of twofold practical relevance as the preventive inoculation with such promoting strains would enhance growth and at the same time prevent the establishment of undesirable relationships between plant and unfavourable strains.
In general data recorded after two months from transfer to ex vitro conditions did not reflect those obtained after one year so indicating that early records are not useful to predict the future plant performances.

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Fungal strain	90 days (%)	120 days (%)	survival (%)
ctrl	48.1 abcd	75.0 bc	70.0
ctrl+ IBA	73.1 bcde	96.1 c	88.0
OMZ	88.5 e	94.2 bc	76.0
OMC	73.1 bcde	86.5 bc	97.4**
OMA	76.9 cde	86.5 bc	94.0**
OME	48.1 abcd	75.0 bc	86.7
OM 89	71.1 bcde	90.4 bc	88.9
OM 90	73.1 bcde	88.5 bc	87.2
OM 91	44.2 abc	78.8 bc	78.7
G1	63.5 bcde	69.2 b	82.6
G2	23.1 a	73.1 bc	73.2
HE	69.2 bcde	76.9 bc	91.7
PS4	36.5 ab	44.2 a	81.1
D8	69.2 bcde	76.9 bc	90.0
D9	36.5 ab	44.2 a	82.7

Tab1- The effect of different fungal strains on: rooting percentage after 90 and 120 days after artificial inoculation and ex vitro plant survival percentage significant difference vs control (as assessed by $\chi 2$ test, $78 \leq N \leq 100$, $P<0.01$).In each column values with different suffix letter significantly differ at the 0.05 level, n=4

Fungal strain	two months (cm)	one year (cm)
ctrl	5.3 e	10.4 a
ctrl+IBA	5.1 de	10.0 a
OMZ	3.4 ab	10.7 a
OMC	4.1 bc	10.5 a
OMA	5.0 de	10.8 a
OME	4.1 bc	11.5 a
OM 89	3.5 ab	11.1 a
OM 90	3.1 a	11.4 a
OM 91	4.4 cd	11.0 a
G1	4.6 cde	10.5 a
G2	3.4 ab	11.4 a
HE	3.9 bc	10.5 a
PS4	3.9 abc	10.8 a
D8	5.2 de	8.8 a
D9	5.1 de	15.5 b

Tab 3 –The effect of different fungal strains on canopy (rosette) diameter in horizontal projection scored after two months and one year after plant transferring to ex vitro conditions. In each column values with different suffix letter significantly differ at the 0.05 level, $19 \leq n \leq 46$

Fungal strain	two months (cm)	one year (cm)
ctrl	3.9 abc	14.3 cde
ctrl+IBA	4.2 abcde	14.7 cdef
OMZ	4.6 bcdef	12.6 abc
OMC	5.1 ef	13.2 bc
OMA	3.9 abcd	16.8 f
OME	4.1 abcd	15.6 def
OM 89	4.9 ef	13.5 bcd
OM 90	5.4 f	11.3 ab
OM 91	4.7 cdef	14.1 cde
G1	3.8 ab	13.2 bc
G2	5.3 f	16.0 ef
HE	3.7 ab	11.7 ab
PS4	4.1 abcd	12.5 abc
D8	3.8 abc	10.5 a
D9	3.7 a	10.4 a

Tab2 –The effect of different fungal strains on plantlet mean length scored after two months and one year after transferring to ex vitro conditions. In each column values with different suffix letter significantly differ at the 0.05 level, $19 \leq n \leq 46$

Fungal strain	two months (n)	one year (n)
ctrl	1.2 a	2.8 abcd
ctrl+IBA	1.2 a	3.2 bcd
OMZ	1.1 a	3.5 d
OMC	1.4 ab	3.6 d
OMA	1.1 a	2.3 ab
OME	1.7 b	3.8 d
OM 89	1.1 a	2.9 abcd
OM 90	1.4 ab	3.5 d
OM 91	1.5 ab	3.5 d
G1	1.2 a	2.3 abc
G2	1.3 ab	3.6 d
HE	1.4 ab	2.8 abcd
PS4	1.3 ab	3.4 cd
D8	1.3 ab	2.0 a
D9	1.4 ab	2.3 ab

Tab 4- The effect of different fungal strains on shoot number assessed after two months and one year after plant transferring to ex vitro conditions. In each column values with different suffix letter significantly differ at the 0.05 level, $19 \leq n \leq 46$